Volume 5 Number 4 April 1978 Nucleic Acids Research

Introduction of ribonucleic acids into cells by means of liposomes

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Received 15 February 1978

ABSTRACT

A method of ultramicroinjection of nucleic acids into cultured cells by means of liposomes is described. Messenger RNA, ribosomal RNA and transfer RNA were entrapped in large unilamellar liposomes and subsequently the liposomes were fused with cells. The uptake of RNA by the cells was stimulated 6 - 8 times by our method. Possible applications of microinjection of RNA by means of liposomes are discussed.

INTRODUCTION

Experimental studies on the interaction of biologically active molecules with cells cultured in vitro are often hindered by the failure of cells to incorporate molecules added to the culture medium. Macromolecules have been introduced into cells by spontaneous uptake (1), by virus-induced fusion of cells with erythrocyte ghosts (2,3), and by microinjection (4,5). The last two techniques are the most efficient currently used. The first of these is quite simple and is applicable to almost all types of animal cells but has the disadvantage of introducing erythrocyte membrane, residual haemoglobin, viral envelopes and inactivated viral RNA, which can have undesirable effects on cells (6). The microinjection method, of introducing material directly into the cytoplasm, leads to little waste of the material, but has the disadvantage of being laborious, particularly when applied to normal-sized cells (4,5).

Recent work has shown that cells in vivo, as well as in vitro, can incorporate large numbers of lipid vesicles (liposomes) without significant cytotoxicity (7,8). Entrapment of water-soluble materials inside such vesicles and subsequent fusion of the vesicles with cells offers a potential method for introducing non-permeable, biologically active molecules directly into the intracellular compartment, without the disadvantages of the previously described methods. In the particular case of introducing ribonucleic acids into cells, the main problem is their degradation by the ribonucleases which

are present in the medium. Entrapment of RNAs in liposomes would protect them from ribonuclease activity and would greatly assist their introduction into the cells. We present here data of entrapment of ribonucleic acids of different sizes, namely reticulocyte ribosomal RNA (rRNA, 28s, 18s, 7s and 5s), globin messenger RNA (mRNA, 9s) and yeast transfer RNA (tRNA, 4s) in liposomes and their introduction into cells in culture.

MATERIALS AND METHODS

Preparation of liposomes containing RNAs.

Large unilamellar liposomes were prepared by dissolving in chloroform 10 μmol of beef brain phosphatidylserine (Lipid Products, Nr. Redhill, Surrey, England) and the solvent was removed by flask evaporation on a rotary evaporator at room temperature. The thin film thus obtained was suspended in NHTE buffer, pH 7.4 (0.1M NaCl, 2mM histidine, 2mM TES, 0.4mM EDTA, pH 7.4) (9). The suspension was vortexed and subsequently sonicated in a bath-type sonicator for 30 min at 30°C under nitrogen. 0.02 mmoles of Ca^{2+} was added and the mixture was incubated for 1 hr at 37°C after which the preparation was centrifuged at 2.500xg for 10 min. The pellet was suspended in 0.1 ml of a solution of RNA which had previously been dialysed against NHTE buffer, pH 7.4. Trace amounts of [^{125}I] RNA were added for quantitating RNA capture inside vesicles. The mixture was vortexed, 0.2 mmoles EDTA was added and the mixture was incubated for 30 min at 37°C. The liposomes were recovered by centrifugation (30,000xg, 20 min at 20°C) and washed with phosphate-buffered saline (PBS). Washed liposomes were suspended in 1 ml PBS, equilibrated for 30 min at room temperature and then passed through a Sepharose 4B column, equilibrated with the same buffer, to separate vesicles containing RNA from untrapped RNA.

Interaction of liposomes with cells.

Mouse L929 cells were grown in minimum essential medium (Eagle) containing 10% heat-inactivated calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin, in 60 mm plastic petri dishes at 37°C in an atmosphere of 5% CO_2 in air. The monolayers were rinsed with PBS and either liposomes (100 nmol of lipid per 10^6 cells) containing [^{125}I] RNA or free [^{125}I] RNA, in 1 ml PBS were added.

Subsequently the cells were incubated at 37°C for 60 min and then the unabsorbed material was removed by washing three times with PBS. The cells on the monolayer were harvested by scraping with a rubber policeman in PBS.

Cells were recovered by a low speed centrifugation in the cold at 200xg for 10 min. The cells were washed two more times with PBS and finally were suspended in 1 ml PBS. 100 µl 50% TCA was added and the whole was held in an ice bath for 30 min. The TCA insoluble material was recovered by centrifugation and after washing with 5% TCA, the radioactivity was measured in a Packard-5230 Auto-Gamma Scintillation Spectrometer.

RESULTS AND DISCUSSION

Capture of RNA by vesicles was determined by the extent of recovery of [^{125}I] RNA in association with vesicles (after chromatography on Sepharose 4B) as a fraction of the [^{125}I] RNA present in the original lipid suspension in buffer and after treatment of liposomes with ribonuclease and chromatography on Sepharose 4B (see below). The liposome fractions were pooled and the radioactivity of TCA insoluble material was measured. Approximately 1.5% of rRNA, 2.5% of mRNA and 5% of tRNA were entrapped in the liposomes. It seems that the amount of entrapped RNA is negatively correlated with the size of the RNA. Preformed liposomes were incubated with [^{125}I] RNA and passed through a Sepharose 4B column to exclude any possibility of non-specific binding of RNA to liposomes. In this case no radioactivity was eluted with the liposome peak (Fig. 1A). To determine if the liposome-entrapped RNAs were protected from ribonuclease activity, the liposome fractions from the Sepharose 4B column were pooled, treated with pancreatic ribonuclease (60 µg/ml, for 60 min at room temperature) and passed again through the same column. Differences in the radioactive pattern before and after treatment with ribonuclease were negligible (Fig. 1B). On the contrary, when the mixture of preformed liposomes and [^{125}I] RNA was treated with ribonuclease, all the radioactivity was eluted in the position of small nucleotides (Fig. 1B). To prove that the entrapped RNAs remain intact, they were extracted from the liposomes and were analysed on formamide polyacrylamide gels. The results in Fig. 2 indicate that RNAs entrapped in liposomes remain intact and they are resistant to ribonuclease activity.

Table 1 shows results of experiments in which mouse L929 cells were incubated with [^{125}I] RNAs, either in solution or entrapped within liposomes. The radioactivity associated with the cells was found to be considerably higher (6-8 fold) when they were incubated with liposome-entrapped RNA than with free RNA at the same concentration. It seems that the size of the entrapped RNA has no effect on the uptake of liposome-entrapped RNA.

Our results indicate that different RNA molecules can be introduced into mammalian cells in culture by means of liposomes. This provides a method

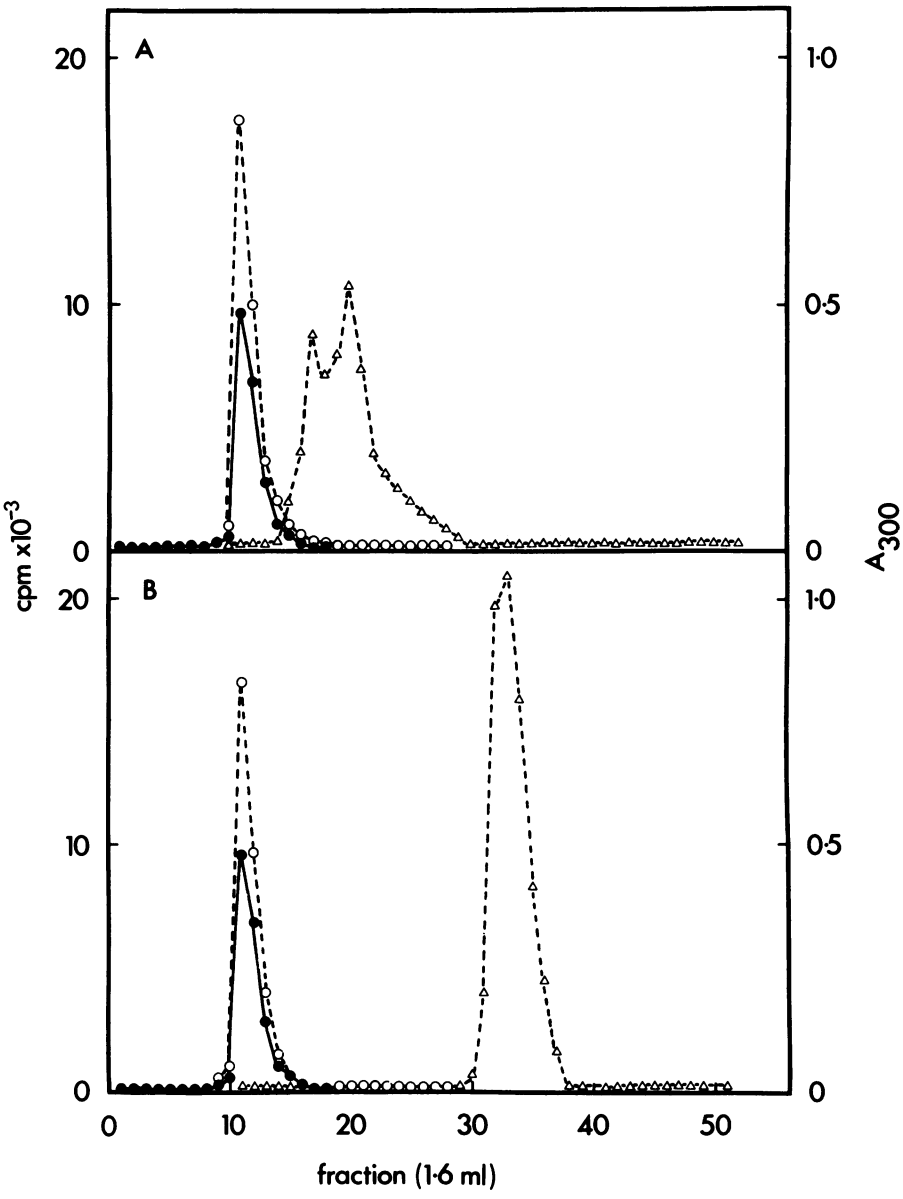

Fig. 1. Entrapment of [^{125}I] rRNA in liposomes. Liposomes were prepared as described in the text. A, before and B, after pancreatic ribonuclease treatment. (o---o) absorbance at 300 nm, (●——●) radioactivity of liposomes containing [^{125}I] rRNA, (Δ---Δ) radioactivity of preformed liposomes incubated with [^{125}I] rRNA. Similar results were obtained when [^{125}I] mRNA or [^{125}I] tRNA were used. Isolation and iodination of RNAs was carried out as described elsewhere (10,11).

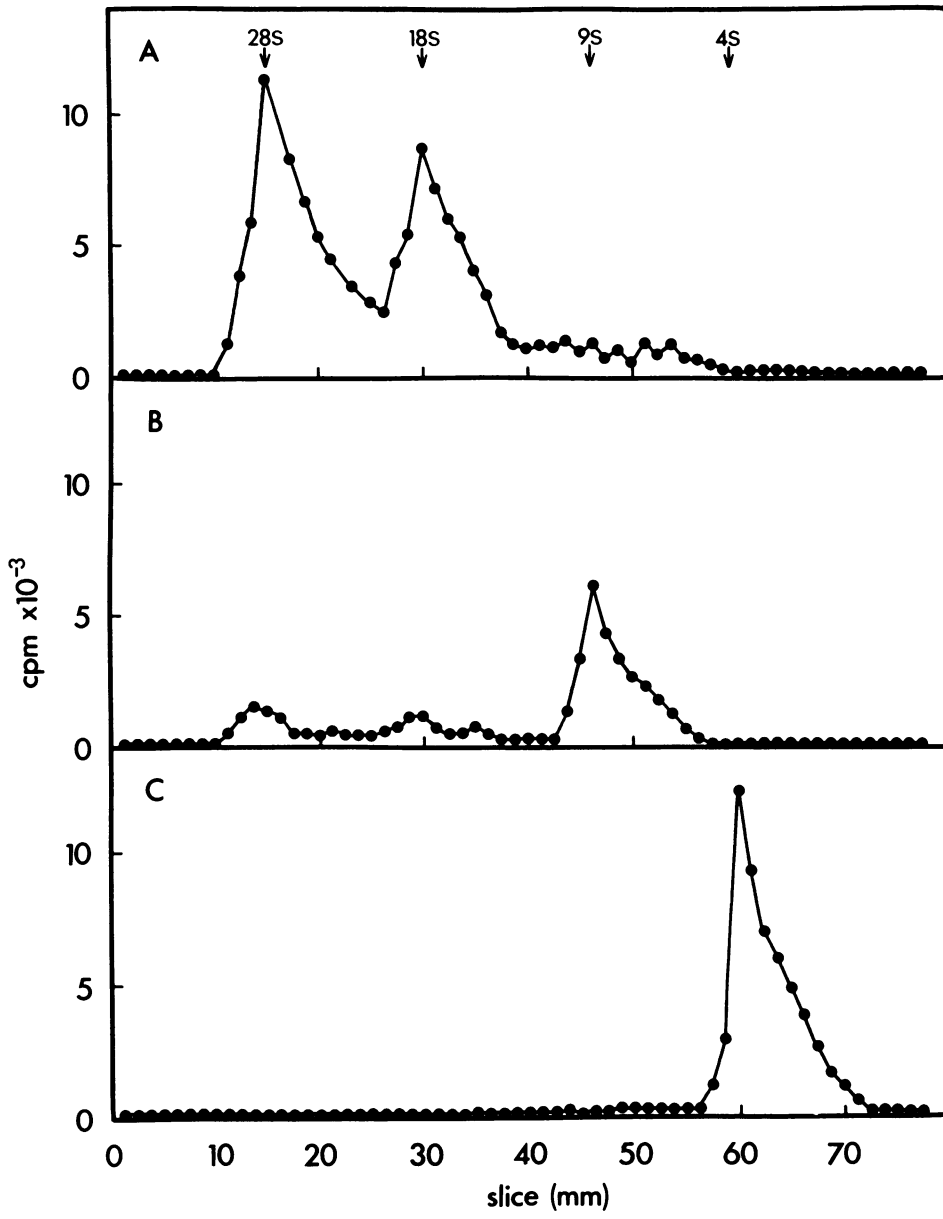

Fig. 2. 4% Polyacrylamide, formamide, gel electrophoresis of RNAs extracted from liposomes. Extraction of RNA, electrophoresis and counting of the gel slices were carried out as described elsewhere (10,11). Unlabelled total reticulocyte RNA and yeast tRNA were used as markers. A, RNA extracted from liposomes containing rRNA, B, containing globin mRNA and C, containing tRNA.

Table 1. Uptake by mouse L929 cells of free and liposome-entrapped [^{125}I]RNAs.

State of [^{125}I]RNA	c.p.m. added	c.p.m. found in liposome treated cells	stimulation of uptake
Free [^{125}I]rRNA	232.510	830	6.85
Entrapped [^{125}I]rRNA	223.740	5.670	
Free [^{125}I]mRNA	263.420	1.220	6.56
Entrapped [^{125}I]mRNA	247.564	8.030	
Free [^{125}I]tRNA	127.320	520	8.15
Entrapped [^{125}I]tRNA	132.810	4.260	

for introducing specific types of RNA molecules (such as suppressor tRNA molecules or messenger RNA molecules) into cells which do not already contain them, to study their interaction with the host cell, as well as problems related to their synthesis and breakdown. Also, by injection of various purified macromolecules, primarily different kinds of messenger RNAs, it would be possible to study the nature and specificity of the translational and post-translational systems of the living cell.

Acknowledgements

G.J.D. received an E.M.B.O. long-term fellowship. I would like to express my thanks to Dr. J.R. Tata for his hospitality and encouragement and to Dr. I. Kerr for his helpful criticism.

REFERENCES

1. Hill, M. and Huppert, J. (1970) Biochim. Biophys. Acta, 312, 26-35.
2. Furusawa, M., Nishimura, T., Yamaizumi, M. and Okada, Y. (1974) Nature, 249, 449-450.
3. Loyter, A., Zakai, N. and Kulka, R.G. (1975) J. Cell Biol., 66, 292-304.
4. Lane, C.D., Marbaix, G. and Gurdon, J.B. (1971) J. Mol. Biol., 61, 73-91.
5. Graessmann, A., Graessmann, M., Hoffmann, H., Niebel, J., Brandner, G. and Mueller, N. (1974) FEBS Lett., 39, 249-251.
6. Furusawa, M., Yamaizumi, M., Nishimura, T., Uchida, T. and Okada, Y. (1976) Methods in Cell Biology (ed. by Prescott, D.M.), 14, 73-80, Academic Press.
7. Gregoriadis, G. (1977) Nature, 265, 407-411.
8. Poste, G., Papahadjopoulos, D. and Vail, W.J. (1976) Methods in Cell Biology, (ed. by Prescott, D.M.) 14, 33-71, Academic Press.
9. Papahadjopoulos, D., Vail, W.J., Jacobson, K., Poste, G. (1975) Biochim. Biophys. Acta, 394, 483-491.
10. Dimitriadis, G.J., Georgatsos, J.G. (1974) FEBS Lett., 46, 96-100.
11. Dimitriadis, G.J., Georgatsos, J.G. (1975) Nucl. Acid Res., 2, 1719-1726.